Transcriptional Regulation of the Intestinal Luminal Na+ and Cl− Transporters

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Abstract

The epithelial apical membrane Na^+/H^+ exchangers (NHE2, NHE3) and Cl^-/HCO_3^- exchangers (DRA and PAT-1) are key luminal membrane transporters involved in electroneutral NaCl absorption in the mammalian intestine. During the past decade, there has been a surge of studies focusing on short-term regulation of these electrolyte transporters particularly for NHE3 regulation. However, the long-term regulation of the electrolyte transporters involving transcriptional mechanisms and transcription factors that govern their basal regulation or dysregulation in diseased states has only now started to unfold with the cloning and characterization of their gene promoters. This review updates the readers with a detailed analysis of the core promoters of NHE2, NHE3, DRA and PAT-1 and outlines the transcription factors involved in their basal regulation as well as in response to both physiological (butyrate, protein kinases, probiotics) and pathophysioloical (cytokines and high levels of serotonin) stimuli. The available information on the transcriptional regulation of the recently identified NHE8 isoform is also highlighted. This review, therefore, bridges a gap in our knowledge of the transcriptional mechanisms underlying the alterations in the gene expression of intestinal epithelial luminal membrane Na^+ and Cl^- transporters involved in electroneutral NaCl absorption. An understanding of the mechanisms of modulation of gene expression of these transporters is important for a better assessment of the pathophysiology of diarrhea associated with inflammatory and infectious diseases and may aid in designing better management protocols.

INTRODUCTION

Electroneutral NaCl absorption is the predominant mechanism underlying Na^+, Cl^- and fluid absorption in the ileum and colon of the mammalian gastrointestinal tract and involves coupling of Na^+-H^+ and Cl^--HCO_3^- exchangers [1–3]. The molecular identity of the proteins involved in this process has only recently become apparent. It is now well established that the luminal membrane sodium hydrogen exchange is mediated via SLC9A2 (NHE2) and SLC9A3 (NHE3) of the SLC9 gene family while the luminal membrane Cl^--HCO_3^- exchange is mediated by SLC26A3 (also termed as DRA, Down Regulated in Adenoma) and SLC26A6 (also termed as PAT1, Putative Anion Transporter 1) of the

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SLC26 gene family [3–6]. Recent studies have also identified the presence of NHE8 as another luminal membrane NHE of intestinal epithelial cells suggested to be important during early life [7]. These proteins specifically NHE2, NHE3, PAT1 and DRA have been well characterized for their functional roles as well as their short-term regulation utilizing in vitro studies [8–13] and knockout mouse models [14–19]. Since the normal function of these transporters is vital to the intestinal fluid absorption and disturbances in their function and expression have been associated with pathophysiology of diarrheal diseases, it is important to understand the molecular basis of the regulation of their gene expression in health and disease. Understanding the long-term regulation of the gene expression of these transporters is also necessary to gain insights into the mechanisms underlying tissue specific and the intestinal region and vertical axis (crypt/villus) expression of these genes. In this regard, the knowledge about the regulation of the gene expression of these important transporters has started to emerge only very recently. To date, however, no comprehensive review detailing the mechanisms of transcriptional regulation of these transporters is available. This review, therefore, focuses on advances in the promoter characterization as well as the mechanisms underlying transcriptional regulation of these transporters under basal conditions and their modulation by physiological and pathophysiological agents.

NA$^+$/H$^+$ EXCHANGERS (NHES)

Members of the Na$^+$/H$^+$ exchange (NHE) gene family are the key transporters involved in the predominant Na$^+$ absorptive pathway in the ileum and proximal colon by mediating electroneutral transport of an extracellular Na$^+$ for a cytosolic H$^+$. Of the nine members of the NHE gene family identified to date, NHE1, NHE2, NHE3, and NHE8 are expressed in the mammalian intestinal tract [20, 21]. NHE1 is localized to the basolateral membrane of intestinal epithelial cells (IECs) where it serves housekeeping functions such as regulation of pH$_i$ and cell volume homeostasis [22]. NHE2 and NHE3 are localized to the apical membrane and play important roles in intestinal Na$^+$ absorption with varying activities in different segments of the GI tract [23]. For example, NHE2 is predominantly expressed in the colon while NHE3 is in the ileum [14, 20, 23]. The importance of NHE3 in Na$^+$ absorption and water homeostasis in the intestine became evident from NHE3 knockout mice, which developed mild diarrhea, low blood pressure and mild metabolic acidosis [24]. Surprisingly, NHE2 knockout mice exhibited no apparent abnormality in intestinal Na$^+$ absorption [16, 25]. NHE8, the recently cloned isoform [21, 26] has been localized to the brush border membranes in both the kidney and IECs [21, 27]. NHE8 is differentially expressed along the human gastrointestinal tract from stomach to colon [28]. However, its functional role in Na$^+$ absorption in adult intestine is not clear at the present.

Studies during the past 2 decades have implicated dysregulation of Na$^+$ absorption and NHE3 down-regulation in various diarrheal disorders including inflammatory bowel diseases (IBD) [29, 30]. The majority of the studies on NHE regulation have focused on the short-term regulation generating a wealth of information on post-translational events of NHE3 regulation involving protein phosphorylation, trafficking, protein turnover, signal transduction pathways, and interaction with other regulatory proteins [8, 9]. However, the long–term regulation afforded by transcriptional mechanisms provides basis for adaptation, and is relevant to disease states or disease intervention as well as understanding the basis for differential expression of NHE isoforms along the length of the intestine. This section highlights our current understanding of the transcriptional mechanisms that impact the expression of NHE2, NHE3 and NHE8, which are expressed on the apical membrane of intestinal epithelial cells. The cloning and characterization of NHE2, 3, and 8 promoters have advanced our knowledge of transcriptional regulation and have provided critical insights into NHE gene regulation.

NHE2

NHE2 Gene—The human NHE2 (hNHE2), encoded by a single copy gene located on chromosome 2q11.2, is composed of 11 introns and 12 exons and occupies ~90 kb of the genome [31]. NHE2 mRNA is expressed at high levels in skeletal muscle, colon, and kidney and at low levels in the testis, prostate, ovary and small intestine, suggesting its tissue-specific expression [32]. The protein product encoded by hNHE2 gene is 812 amino acids long and when expressed in an *in vitro* cell-free system shows a band of ~75 kDa [32], representing the unglycosylated immature form, while the mature form expressed in human intestinal C2BBE1 cells is of ~85 kDa [33].

NHE2 Promoter—To date human and rat NHE2 promoters have been cloned [31, 34]. Transcription initiation site (TIS) of hNHE2 was localized to the adenine residue 316 nucleotides upstream from the ATG translation start codon. Comparison of the nucleotide sequences of the cloned region with the promoter databases revealed the presence of numerous potential transcription factor-binding sites including Sp1, Egr-1, AP-2, GATA, NFκB, Oct-1, Cdx-2 and a number of half-sites for glucocorticoid and thyroid hormone receptors, suggesting that regulation of this gene involves a complex array of regulatory factors [31] (Figure 1). The human and rat NHE2 promoter sequences exhibited an overall 59% sequence homology with several conserved binding sites for transcription factors AP-2, Sp1, Egr-1, CACCC, NFκB and Oct-1 [31]. There were also unique motifs for other transcription factor binding sites suggesting that distinct mechanisms may also be involved in regulation of these orthologs [31]. The human and rat NHE2 promoters both lack TATA- and CAAT-boxes. As typical of all other TATA-less and CAAT-less promoters, the NHE2 promoter is highly GC-rich and contains multiple GC-boxes. These motifs are targeted by Sp1 transcription factor family members as well as other transcription factors that show high affinity for GC-rich sequences, such as Egr-1, Krüpple-like factors, ZBP-89 and MAZ [35, 36].

NHE2 Core Promoter Region—Functional characterization of 5′-deletion constructs of the hNHE2 promoter localized the core promoter region to nucleotides −40 to +150 bp (Figure 1), suggesting that cis-elements required for maximal basal transcriptional activity are located in this region. Further 5′-deletions, which caused 70% reduction in promoter activity, suggested the critical role of GC-elements contained in the deleted region (−40 to −20 bp) in promoter regulation. EMSA and ChIP assays demonstrated Sp1 and Sp3 transcription factors to bind to GC-element centered at −25 bp region [37, 38]. Competition and mutational analyses identified the Sp1-b and Sp1-c binding sites to be essential for interaction with Sp1 and Sp3 and also suggested a cooperative role between Sp1-b/Sp1-c and downstream sequences for maximal NHE2 promoter activity [38] (Figure 1). The Initiator (Inr) element, a functional analog of TATA-box, in conjunction with or without a TATA-box can promote transcription initiation [39]. The sequences surrounding NHE2 transcription initiation site exhibit a weak homology to Inr consensus sequence [40] and were found to interact with transcription factors Sp1 and Sp3 [38]. In addition to Sp1 [14], other proteins such as USF1 [41], YY1 [42] may also interact with the Inr element and enhance transcription. A USF binding site is present in the 5′-UTR of the hNHE2 promoter. Deletion of this site diminished basal NHE2 promoter activity [38].

Regulation of NHE2 by Sp1 and Sp3—Sp1 binds with high affinity to consensus GC-box elements 5′-G/TGGGCGGG/AG/AC/T-3′ [43, 44]. Consistent with the involvement of Sp1 and Sp3 in the regulation of basal NHE2 transcription, multiple binding sites for Sp1 have been identified in both the human and rat NHE2 promoters [31, 34, 38, 45, 46]. The importance of Sp1 and Sp3 in promoter activity of NHE2 was also shown in cotransfection assays in *Drosophila* SL2 cells, which are devoid of endogenous Sp1 related factors [47].

Both Sp1 and Sp3 were capable of trans-activating NHE2 promoter and Sp3 stimulated NHE2 to a greater extent than Sp1 [38].

In contrast to the stimulatory effects of Sp1 or Sp3 on hNHE2 promoter in intestinal epithelial cells, Sp3 inhibited while Sp1 stimulated rNHE2 transcription in renal cells [45]. While the rNHE2 minimal promoter (bp −36/+116) was actively expressed in the mouse renal cell line mIMCD-3, it was insufficient to drive transcription in either the rat intestinal epithelial cells (RIE) or the human intestinal cell line (Caco-2) [46]. A larger rNHE2 promoter fragment (−69/+116) was required for basal promoter activity in RIE cells. Similar to hNHE2 promoter, both Sp1 and Sp3 displayed positive effects on rNHE2 promoter activity in intestinal epithelial cells, whereby, Sp3 exhibited higher transactivation potential than Sp1. The lack of promoter activity of the rNHE2 −36/+116 bp region in intestinal epithelial cells may stem from cell-specific expression (mIMCD-3 vs. human intestinal C2BBE1 cells), as species differences did not appear to be involved. Regulation of the human and rat NHE genes by Sp1/Sp3 appears to be mediated through distinct response elements [38, 46]. This is evident by functional diversity of Sp1 binding sites in the human −40 to +150 bp and rat −36 to +116 bp promoter region. Furthermore, the rat Sp1 elements at −69 to −36 bp, which are responsible for its expression in the intestinal epithelial cells are not conserved in the human NHE2 promoter sequence [31]. Based on the current data, the basal regulation of NHE2 appears to involve various Sp1 binding sites.

NHE3

NHE3 Gene—The human NHE3 gene, located on chromosome 5p15.3 [48], is expressed in a tissue-specific manner. Northern blot analysis detected hNHE3 mRNA in decreasing amounts in following order: kidney > small intestine > testes > ovary > colon   prostate > thymus > peripheral leukocyte   brain > spleen > placenta. The cDNA encodes for a protein of 834 amino acids with calculated molecular mass of 92,906 [49]. A single transcription initiation site (TIS) was identified 116 nucleotides upstream from the hNHE3 translation start site [50]. The genomic organization of the hNHE3 gene is not fully characterized. However, in silico analysis of hNHE3 cDNA sequence revealed that the hNHE3 gene spans >50 kb of the chromosome 5 and contains 17 exons and 16 introns (Malakooti, unpublished data). The organization of exons and introns of the hNHE3 gene is similar to the reported rNHE3 gene [51, 52]. The genomic organization of NHE8 has not been defined.

NHE3 Promoter—hNHE3 promoter was characterized by cloning a ~3.2 kb human genomic DNA spanning from −3117 to +131 bp [50]. The nucleotide sequence of 1.5 kb of the 5′-flanking region from (TIS), which contained an atypical TATA-box and multiple GC-elements that contained the overlapping binding sites for Sp1 as well as Egr-1, AP-2 and CACCC [50] Further upstream, putative binding sites with relevance to the regulation of intestinal genes or tissue-specific and developmentally controlled genes, such as Cdx-2, Sp1, AP-2, USF, MyoD, cAMP-response element binding site (CREB), VDR, T3R, half-sites for GRE, and CACCC were identified [50] (Figure 1).

The rNHE3 promoter was cloned by two different groups and potential transcription factor binding sites were reported [51, 52]. A comparison of the nucleotide sequences of the human and rat NHE3 promoters revealed an overall sequence homology of 38%, whereas, significant conservation of the nucleotide sequences (79%) and transcription factor binding sites were found within the proximal promoter region [50]. Both the human and rat NHE3 promoter regions lack an authentic TATA-box and CAAT-box in close proximity of the TIS. Furthermore, this region is highly GC-rich in both promoters, which is typical of many developmentally and differentially regulated gene promoters [39]. The consensus Sp1, AP-2,

CACCC, and the atypical TATA-like sequences appeared at the same positions in both species in this region.

NHE3 Core Promoter—Deletion analyses of the hNHE3 promoter-reporter constructs and expression in C2BBE1 cells identified the core promoter region that extends from −88/+5 bp and confers maximal promoter activity [50, 53]. The promoter activity was reduced by 60% with further deletion to −76 bp. The sequences downstream from −76 bp represents the minimal promoter, which confers ~40% of the maximal basal promoter activity [50, 53] (Figure 1).

Regulation of NHE3 by Sp1 and Sp3—The basal activity of the hNHE3 promoter is driven by the functional overlapping Egr-1/Sp1 motif contained within the −89/−69 bp region and overlapping Sp1/AP-2/Sp1 sequences at −72/−49 bp. Deletion or disruption of these motifs significantly decreased the promoter activity [50, 53]. DNA-binding assays demonstrated that the Egr-1/Sp1 element at −89/−69 bp binds to Sp1 and Sp3 at basal growth conditions. This motif in conjunction with downstream cis-elements confers maximal transcriptional activity to the hNHE3 promoter. This site is of central importance to the basal as well as regulated expression of NHE3 promoter [50, 53–56].

The Sp1/AP-2/Sp1 element at −72/−49 bp of the hNHE3 promoter was shown to bind AP-2 [50]. The critical role of Sp1 and Sp3 in the basal expression of hNHE3 was demonstrated by siRNA knockdown of these transcription factors in C2BBE1 cells that caused dramatic decrease in the basal hNHE3 promoter activity [54]. Further, transactivation of hNHE3 promoter by Sp1 and Sp3 was confirmed by exogenous overexpression of Sp1 and Sp3 in SL2 cells [53]. Kiela et al. [57] investigated the mechanism of rNHE3 gene regulation in transiently transfected Caco-2, IEC-6, QT6, and *Drosophila* SL2 cells. Deletion and mutational analysis showed that the atypical TATA element in the proximal promoter region did not play a role in the transcriptional activation of rNHE3 promoter [57]. Three potential Sp1 motifs in promoter region downstream from −81 bp were shown to be critical for rNHE3 promoter activity. It was also shown that Sp1 and Sp3 interact synergistically with a GATA-5 site in the exon-1 leading to enhanced promoter activity, which may be important for a gradient of intestinal NHE3 expression along the crypt-villus axis [57]. The hNHE3 promoter lacks the GATA motif in the 5′-untranslated region (5′-UTR). However, exon-1 sequence harbors two sets of direct repeat elements [50] and a 7-amino acid long minicistron [49]. These elements could be involved in downregulation of NHE3 gene expression, as deletion of this 5′-UTR resulted in a moderate increase in hNHE3 promoter activity [50].

NHE3 expression in cultured cells has been shown to be increased during cell confluence in a STAT-3-dependent manner, which appeared to be critical for dome formation in polarized epithelial cells [58]. Furthermore, a cooperative interaction between STAT-3 and Sp1/Sp3 has been shown in this effect of STAT3 on NHE3 expression [58]. This further emphasizes the importance of Sp1/Sp3 in NHE3 expression during cell confluence in polarized epithelial cells.

NHE8

NHE8, the newly identified apical membrane Na^+/H^+ exchanger, which was initially cloned from mouse kidney cDNA library [26]. The rat NHE8 cDNA encodes for a protein of 575 amino acids residues. The protein product of this gene was detected at 65 kDa in BBM preparations from the rat jujenal mucosa by Western blot analysis [21, 59, 60]. The genomic organization of NHE8 gene has not been characterized.

NHE8 is present intracellularly in most cells [61], but also in the apical membrane of epithelial cells of intestine [21, 59, 60] and kidney [26], serving as Na^+/H^+ exchanger [28].

Contrary to the expression levels of NHE2 and NHE3 during development, NHE8 mRNA expression was higher in 2–3 -week old rats and lower in adult rats [21, 59, 60]. This suggests an important role of NHE8 in the early stages of development.

NHE8 Promoter—The human NHE8 promoter was recently cloned [62]. The transcription initiation site was mapped to a Guanine residue 95 bp upstream from the translation initiation codon by 5′-RACE analysis. Characterization of the promoter region localized the minimal promoter to a fragment spanning −32 /+17 bp with respect to TIS, which interacts with Sp3 [62].

MODULATION OF NHE GENE EXPRESSION

The following sections highlight the mechanisms underlying the modulation of NHE gene expression by physiological and pathophysiological agents (Table 1).

Physiological Stimuli

Butyrate—Butyrate, a short chain fatty acid, is produced in the colon by microbial fermentation of dietary fiber. Butyrate exerts diverse effects on cellular functions and has been shown to regulate cell proliferation, differentiation, and apoptosis [63]. Butyrate is also implicated in suppressing inflammation and carcinogenesis [64]. It enhances the colonic NaCl absorption [65, 66], and butyrate-dependent increase in Na^+ absorption was demonstrated to be mediated by both NHE2 and NHE3 in rat distal colon [66]. Musch et al. [67], demonstrated that butyrate enhanced NHE3 activity in C2BBE1 cells via elevated expression of NHE3 mRNA and protein. Further, *in vivo* studies with fiber-supplemented rats for 2-days displayed increased $^{22}Na^+$-uptake in colon secondary to enhanced NHE3 mRNA and protein levels [67]. These findings indicated transcriptional activation of NHE3 by butyrate as the likely mechanism for NHE3 response to butyrate. Amin et al. [55], demonstrated that butyrate-induced enhancement of hNHE3 mRNA expression and promoter activity in C2BBE1 cells required Sp1 and Sp3 binding to the NHE3 core promoter region (−89/−69 bp). siRNA silencing and studies utilizing pharmacological inhibitors of specific protein phosphatases further suggested that dephosphorylation of Sp1/Sp3 in response to butyrate may play a role in their enhanced binding affinity to the hNHE3 promoter and stimulation of NHE3 by sodium butyrate.

Sodium butyrate also activates rNHE3 gene promoter via a ser/thr kinase-dependent mechanism [68] and involved response elements within −320/−34 bp of the rNHE3 promoter. Subsequent studies identified Sp1 binding sites at −58/−55 bp as the critical cis-elements for sodium butyrate induced rNHE3 promoter activity. Sp1 and Sp3 were found to interact with this motif at basal growth conditions, however, DAPA studies with butyrate treated nuclear proteins showed decreased Sp1 and increased Sp3 protein co-precipitation with this motif. Furthermore, butyrate treatment led to increased phosphorylation of Sp1 and acetylation of Sp3, with the later suggested to be involved in mediating the effects of butyrate on the rNHE3 promoter activity [69]. To date, no detailed studies are available on the effects of butyrate on the NHE2 or NHE8 gene expression.

Glucocorticoids—Glucocorticoids increase intestinal salt and water absorption and increase NHE3 activity and mRNA abundance in the intestine and kidney [70, 71]. Methylprednisolone, a commonly used treatment for inflammatory bowel diseases, has been shown to reverse the inhibition of electrolyte and nutrient transport in animal models of IBD or patients with Crohn's disease and ulcerative colitis [72, 73]). Putative cis-elements for half-sites for GRE, were identified in the 5′-flanking region of hNHE3 promoter [50]. These glucocorticoid response elements (GRE) were also found in the rNHE3 promoter and dexamethasone, a synthetic glucocorticoid, was shown to activate rNHE3 promoter in

transiently transfected OK and LLC-PK1 renal cells [51, 52]. Studies by Yun et al. [70], showed that long-term treatment of rabbits with methylprednisolone resulted in enhanced NHE3 mRNA levels in the ileum, whereas NHE1 and NHE2 expression did not change [70]. These data suggested that glucocorticoid effects on NHE3 was mediated by a transcriptional mechanism.

In contrast, recent studies showed that NHE8 expression was decreased in response to glucocorticoids in jejunal and ileal mucosa from rats and in Caco-2 cells [74]. Functional analyses of the hNHE8 promoter in Caco-2 cells localized the GRE to −89/−32 bp region. Site-directed mutagenesis and EMSA demonstrated that Pax-5, a member of the paired box family of transcription factors, interacts with the promoter region at −75/−50 bp and represses the promoter activity [74].

Epidermal Growth Factor—EGF administration to rats has been demonstrated to increase NHE2 mRNA in jejunal mucosa and also enhance NHE2 activity in BBMV prepared from rat jejunum. [75]. Similar results were obtained in vitro in the rat intestinal epithelial cells (RIE). Furthermore, actinomycin D, a transcription inhibitor, blocked not only NHE2 mRNA expression, but also EGF-induced NHE2 activity indicating that a transcriptional mechanism is likely responsible for EGF effect on NHE2 activity. Consistently, EGF also enhanced rNHE2 promoter reporter activity in transiently transfected RIE cells [75]. Similarly, the hNHE2 transcription was also stimulated by EGF [33]. In contrast, EGF reduced the hNHE8 mRNA expression and promoter activity in Caco-2 cells [76]. EGF-mediated repression of NHE8 promoter activity in Caco-2 cells was shown to involve MAP kinases ERK1/2 and Sp3 DNA-binding.

Phorbol 12-myristate 13-acetate—PMA, a diacylglycerol analogue, modulates diverse cellular functions through stimulation of signaling pathways such as protein kinase C (PKC) and mitogen-activated protein kinases (MAPK). Long-term exposure to low concentration of PMA was shown to stimulate the expression of both NHE2 and NHE3 mRNA and enhance their promoter activity in C2BBE1 cells [37, 53]. Functional analyses and DNA-binding assays showed localization of the PMA response elements (PRE) to the −339/ 324 bp region of NHE2 and −89/ 69 bp of NHE3 promoters (Figure 1). The PRE, 5′-GCGGGGGCGGG-3′, were composed of overlapping binding sites for Egr-1 and Sp1 transcription factors, which were also identified in the promoters of other PMA target genes [77, 78]. DNA binding assays showed Sp1 and Sp3 in the basal growth conditions and Egr-1 in PMA-treated nuclear extract interact with PRE of both NHE2 and NHE3 promoters [37, 53]. Binding of PMA-induced Egr-1 to the NHE2 and NHE3 PREs eliminated the Sp1/Sp3 interactions with these motifs indicating that newly synthesized Egr-1 could displace the prebound Sp1/Sp3 and interact with the overlapping Egr-1/Sp1 motif. Egr-1 overexpression studies showed that Egr-1 was sufficient to induce NHE2 and NHE3 promoter activities. siRNA targeted to Egr-1 diminished the stimulatory effects of PMA on NHE3 mRNA expression [37, 53].

Pathophysiological Stimuli

Inflammatory mediators—The production of various proinflammatory cytokines such as IFNγ, TNFα, and IL-1β in the intestine is one of the major contributing factors to the pathogenesis of inflammation-associated diarrhea in IBD. The expression and activity of NHE3 has been shown to be suppressed by colonic inflammation, [79, 80] and proinflammatory mediators TNFα and IFNγ [81]. Rocha et al. [81] demonstrated that both in rats and C2BBE1 cells, the expression and activity of NHE2 and NHE3 were downregulated by IFNγ. Recently, it has been shown that simultaneous addition of both TNFα and IFNγ resulted in significant reduction of NHE3 promoter activity that involved

the 95/+5 bp region of the NHE3 promoter [54]. TNFα/IFNγ treatment led to diminished binding of Sp1/Sp3 to the Egr-1/Sp1 motif at −89/−69 bp, while the level of Sp1/Sp3 protein expression was not altered. Sp1/Sp3 binding to NHE3 proximal promoter region was also confirmed by ChIP assays [54]. The inhibitory effect of TNFα/IFNγ on the NHE3 promoter was completely blocked by inhibitors of PKA, implicating cytokine-dependent activation of PKA in the repression of NHE3 [54]. Sp1 is known to be phosphorylated by different protein kinases including PKA [79] and dephosphorylated by protein phosphatases [82]. In this study, binding affinity of Sp1 and Sp3 proteins for NHE3 promoter region was found to be significantly decreased after *in vitro* phosphorylation of nuclear proteins by α-catalytic subunit of PKA [54]. These results suggested that Sp1/Sp3 were essential for NHE3 promoter activity and their phosphorylation by PKA may be involved in repression of NHE3 transcription by cytokines (Figure 2). Along the same lines, butyrate was found to restore the cytokine-induced inhibition of the NHE3 transcriptional activity and mRNA expression [55]. These findings are important as restoration of the NHE3 function in conditions associated with decreased NHE3 activity could have therapeutic relevance if defective Na$^+$ absorptive process could be corrected. A recent study by Yeruva et al. [83], found no significant difference in NHE3 mRNA and protein abundance, but NHE3 function was reduced in UC patients. However, this is in contrast to earlier studies showing decreased NHE3 mRNA and protein levels in UC [84]. These data suggest that transcriptional regulation, in part, may be responsible for impaired Na$^+$ absorption in IBD.

The NHE2 mRNA and protein levels as well as its activity is repressed by long-term exposure to IFNγ [81] in the rat intestine and C2BBE1 cells and TNFα [33] in C2BBE1 cells. Studies utilizing actinomycin D plus TNFα further confirmed transcriptional regulation of NHE2 [33]. Consistent with these data, NHE2 promoter activity was also diminished by TNFα treatment. Functional characterization of the 5′-regulatory region of the NHE2 promoter localized the TNFα responsive region and identified an NFκB motif as the TNFα response element (−578 to −568 bp) [33] (Figure 1). TNFα-mediated repression of NHE2 mRNA was accompanied by reduced NHE2 protein abundance as well as its activity (Figure 2).

NHE8 mRNA and protein levels were also reduced in jejunal mucosa from TNBS or LPS-treated rats [62] suggesting that this isoform is also down-regulated by inflammation. In vitro studies in Caco-2 cells demonstrated TNFα-induced downregulation of hNHE8 promoter activity via reduction of DNA-binding activity of Sp3 [62]. Reduced expression of NHEs in inflammation suggests that this may contribute to the diarrhea associated with inflammatory conditions.

Serotonin—Serotonin (5-HT), an important neurotransmitter predominantly present in the gut and brain, plays a critical role in motility, secretion and absorption in the gut [85]. Increased levels of serotonin in the gut have been implicated in pathogenesis of diarrhea associated with IBS and UC [86]. Under acute conditions serotonin decreases NHE2 and NHE3 activities in Caco-2 cells [87]. Long-term treatment of C2BBE1 cells with 5-HT resulted in a significant reduction in NHE3 mRNA and protein expression as well as its promoter activity [56]. This reduction in promoter activity appears to be due to reduced DNA-binding activity of Sp1/Sp3 transcription factors to the −89/−69 bp in the NHE3 proximal promoter region. Negative impact of serotonin on NHE3 could be blocked by PKCα antagonist, Gö6976, implicating PKCα pathway in regulation of the repressive effects of serotonin on NHE3 [56]. To date, no data is available on the long-term effects of serotonin on the NHE2 and NHE8.

CL⁻/HCO₃⁻ EXCHANGERS

Studies over the past decade have shown that DRA and PAT1 are the candidate genes mediating intestinal luminal Cl⁻/HCO₃⁻ exchange [3, 6]. DRA, identified as a gene downregulated in colonic adenomas [88] was shown to be the major Cl⁻/HCO₃⁻ exchanger involved in electroneutral NaCl absorption in the intestine [12, 19]. Mutations in hDRA have been shown to result in congenital chloride diarrhea (CLD), an autosomal recessive disorder characterized by a watery stool with high chloride concentration and metabolic alkalosis [89–91]. Similarly, the phenotype of DRA knockout mice was similar to CLD in humans [17]. PAT-1 knockout mice showed a decrease in apical Cl⁻/HCO₃⁻ exchange activity but did not exhibit diarrheal phenotype [92]. It appears that while PAT-1 is involved in mediating Cl⁻ absorption, it is not directly coupled to the water movement as seen with DRA.

Studies have shown that expression of both DRA and PAT-1 varies along the length of the intestine [19, 93] and crypt-villus axis [93–96]. DRA protein is predominantly expressed in the colon compared to much lower levels in the small intestine [3, 19, 93] and is localized to the apical membranes of IECs [19, 90, 96]. However, the expression pattern of PAT-1 is opposite to that of DRA expression in the intestine. PAT-1 is abundantly expressed in the small intestine compared to the colon [95], suggesting that PAT1 is important for the apical Cl⁻/HCO₃⁻ exchange in the small intestine, while DRA is important in the colon [3]. The protein product encoded by DRA gene is a glycoprotein of 764 amino acids [97]. PAT-1 gene encodes an integral membrane protein of 738 amino acids [98]. With respect to the crypt-villus/surface axis distribution, in situ hybridization studies showed expression of DRA in the surface epithelium and upper crypt of the rat and human colon [96]. These observations correlate well with the recent studies showing that DRA Cl⁻/HCO₃⁻ exchange activity was higher in the upper region (surface) of the colonic crypts compared to the lower region (middle & base) [93]. However, PAT-1 was expressed at very low levels in the crypts [95]. Ontogenic studies in mice showed that DRA mRNA expression in colon is low at birth, but increases during postnatal development [99]. However, the small intestine DRA mRNA expression remained lower throughout the postnatal development [99]. Similarly, well-differentiated post-confluent Caco2 cells showed higher expression of DRA with no expression in pre-confluent cells [99]. The above studies suggest that regional, crypt-villus axis or ontogenic expression of DRA and/or PAT1 in the intestine must be under transcriptional regulation. However, very limited studies are available regarding transcriptional regulation (long-term) of DRA and PAT-1 via changes in gene transcription. For example, a few studies have shown an inhibition of DRA expression in intestinal inflammation and in response to *C. rodentium* infection [93, 96, 100]. PAT-1 expression was also decreased in response to the pro-inflammatory cytokine, IFNγ [101]. In contrast, DRA expression was shown to be increased in response to anti-inflammatory/pro-absorptive agents such as probiotics [102] and butyrate [103]. New insights into the transcriptional mechanisms of DRA and PAT1 regulation are beginning to emerge with the cloning and characterization of the promoter regions of the human DRA and PAT1. The following sections will discuss in detail: 1) the basal characterization and regulation of DRA and PAT-1 promoters; and 2) the transcriptional regulation of DRA & PAT-1 under both physiological and pathophysiological conditions.

DRA

DRA Gene—DRA gene consists of 21 exons and is localized to chromosome 7q22-q31.1 [104]. The DRA cDNA encodes for a protein of 764 amino acids with a predicted molecular weight of 85 kDa [104].

DRA Promoter

A 3765 bp of the 5′-regulatory region of DRA gene (promoter) was cloned by hybridization screening of a human bacterial artificial chromosome (BAC) genomic library [103]. DRA promoter (p-3765/Luc) was found to be significantly active in LS174T colonic cells [103] and intestinal Caco2 cells [100]. However, in the non-intestinal cells, HepG2 & NIH3T3 fibroblasts, p-3765/Luc DRA promoter construct showed no activity indicating that DRA promoter activity is intestinal cell line dependent. Sequence analysis of the DRA promoter and comparison with transcription factor databases revealed the presence of potential binding sites for transcription factors such as AP1, c-Jun & HNF4, GATA, YY1 and STAT1 (Figure 3).

Core Promoter Region

Luciferase reporter assays of the progressive 5′-deletions of hDRA promoter suggested that cis-elements required for basal transcriptional activity are located in the DNA region between −398 and −102 bp [103]. Sequence analysis of the core promoter region indicated the presence of potential binding sites for transcription factors such as AP1, c-Jun & HNF4 (Figure 3). DNase I footprint analysis and EMSA showed HNF4 may be important in driving the basal activity of DRA promoter in LS174T human colonic cells [103]. HNF4 binds to the consensus sequence 5′-AGGTCANAGGTCA-3′ in order to activate transcription. The role of AP1 & c-Jun in the basal regulation of DRA promoter activity has not been studied and future studies are needed to elucidate their involvement in the basal regulation of DRA promoter in detail. The physiological significance of regulation of DRA promoter activity was further validated in an in vivo mouse model [103]. Expression of human growth hormone (hGH) driven by DRA promoter in transgenic mice was seen specifically in the villus epithelial cells of the small intestine and in surface epithelial cells of the colon [103] but not in other non-intestinal tissues suggesting that expression of DRA is specific to the intestine and differentiation status of the epithelium.

PAT1

PAT-1 Gene—PAT-1 gene is composed of 21 exons, 21 introns and spans 9.7 kb of chromosome 3p21.3 [105]. SLC26A6 gene encodes an integral membrane protein of 738 amino acid with a predicted topology of 11 transmembrane helices and an intracellular –NH2 and -COOH terminus [98, 105]. Human SLC26A6 gene was also found to have three alternatively spliced variants, named SLC26A6 a, c and d. With regard to their tissue distribution, RT-PCR studies indicated that SLC26A6a but not c or d is the spliced variant expressed in the human small intestine and colon [98]. Human PAT1 isoforms a, c and d comprise of 12, 8 and 12 membrane spanning domains, respectively.

PAT-1 Promoter—The 5′-flanking region of PAT-1 gene was cloned by PCR utilizing human genomic DNA and gene specific primers [101]. TIS was localized to Guanine residue 99 nucleotides upstream from the ATG translation start codon. Studies showed that PAT-1 promoter activity (1120 bp; p-964/+156 bp) was high in Caco2 cell line (which on differentiation exhibits small intestinal phenotype) compared to colonic NCM460 and kidney HEK 293 cells consistent with the fact that PAT-1 is predominantly expressed in the small intestine [95]. Sequence analysis of PAT1 promoter showed consensus sites for various transcription factors such as HNF1α, YY1, Sp1, CdxA, AP-2, Mzf1, MyoD & Egr1 (Figure 3).

Core Promoter Region—Progressive deletions from the 5′-flanking region demonstrated that deletions from −714 to −214 bp did not exhibit any change in promoter activity compared to full length PAT-1 promoter construct (−964/+156). Further deletion to −44 bp

significantly decreased promoter activity by ~80% indicating that the region between −214 and −44 bp harbors cis elements important for the basal activity of PAT-1 promoter [101]. Sequence analysis of this region revealed the presence of potential binding sites for HNF1α, YY1 & Sp1 (Figure 3). More detailed studies are needed to further define the regulation of PAT1 by these transcription factors. Besides human, there are no reports on the cloning & characterization of DRA or PAT1 promoters in other species.

MODULATION OF DRA AND PAT1 GENE EXPRESSION

Long-term regulation of gene expression generally mimics the prolonged physiological or pathophysiological changes that occur in the intestine such as in response to luminal SCFA butyrate, or intestinal diseases (e.g. IBD). The following section will focus on the transcriptional regulation of DRA & PAT1 gene expression in response to both physiological and pathophysiological stimuli (Table 1).

Physiological Stimuli

Butyrate—Recent studies have shown that butyrate stimulates DRA expression and promoter activity in LS174T colonic cells [103]. Progressive 5′-deletions of DRA promoter showed that the butyrate-responsive region was located between −688 to −398 bp region. DNase I footprint analysis and EMSA identified a region from −468 to −487 bp that harbored two overlapping potential binding sites for YY1 and GATA indicating their involvement in butyrate-induced stimulation of DRA promoter activity [103]. Competition studies showed that YY1 consensus oligonucleotide completely abolished YY1 binding and also decreased GATA binding. On the other hand, GATA consensus oligo did abolish GATA binding but increased YY1 binding indicating that YY1 and GATA may influence the binding of each other. Mutations in the potential YY1 & GATA binding sites attenuated the stimulatory effects of butyrate on promoter activity further suggesting that the binding of YY1 and GATA transcription factors is crucial for butyrate-induced stimulation of DRA promoter [103]. Interestingly, YY1 binding in the DRA promoter was competed by a 20 bp fragment from the promoter region of fatty acid binding protein *fabp* gene that was previously known to silence the expression of FABP in the crypt epithelium of the small intestine [106]. It seems that YY1 may be critical for silencing the expression of DRA in proliferating undifferentiated intestinal epithelial cells and may play an important role in determining their expression pattern along the crypt-surface axis in the colon. GATA-5 was shown to modulate the promoter activity of rat NHE3 and suggested to play a role in its differential expression along the crypt-villus axis [57]. The GATA isoform involved in butyrate-induced effects on DRA promoter activity is not known [103]. Future studies are needed to elucidate the interplay of GATA & YY1 and to identify the isoform of GATA involved in butyrate-induced effects on DRA promoter activity. Also, regulation of PAT-1 gene expression by butyrate is not known.

Lactobacillus acidophilus—*Lactobacilli* are one of the predominant commensal bacteria in the gut microflora and have previously been used for the prevention and treatment of diarrheal disorders [107]. Recent studies utilizing Caco2 monolayers have shown that *Lactobacillus acidophilus* (LA) stimulated DRA mRNA and protein expression [102]. However, PAT-1 mRNA & protein expression remained unaltered upon LA treatment. In vivo mouse studies also demonstrated a significant increase in DRA mRNA and protein levels [102] in response to LA. Culture supernatants (CS) of live LA bacteria showed similar effects as live bacteria and stimulated both DRA expression and function in Caco2 monolayers [102]. The effects of LA CS were also seen at the transcriptional level. LA CS significantly increased DRA promoter activity in Caco2 cells [102]. These studies suggested that stimulation of DRA promoter activity and expression by LA secreted soluble effector

molecules may contribute to the upregulation of intestinal Cl⁻ absorption and provide novel insights underlying thepotential antidiarrheal effects of LA. However, the molecular mechanisms involved in the transcriptional regulation of DRA by LA secreted soluble factors are not known. Further studies are needed to identify the cis elements and transcription factors involved in the regulation of DRA promoter activity by LA secreted soluble factors and to elucidate the identity of soluble factors involved in the stimulation of DRA promoter activity.

Pathophysiological Stimuli

Inflammatory Mediators—Recent studies have demonstrated that chloride absorption is reduced in patients with ulcerative colitis [93]. The reduction in Cl⁻ absorption in the surface of the colonic crypts was consistent with a decrease in DRA mRNA expression in UC patients compared to normal [93]. A decrease in DRA mRNA expression has also been reported in the cecum and colonic regions of two different animal models of colitis: IL10 knockout mice and HLA-B27/β2M transgenic rat [96]. *In vitro* studies in response to the pro-inflammatory cytokines utilizing Caco2 cells have also shown a decrease in DRA expression by IL1β [96] and a decrease in both DRA & PAT-1 expression by IFNγ [100, 101]. The transcriptional mechanisms underlying repression of DRA and PAT1 by IFNγ have been investigated in detail as outlined below.

IFNγ elicits its effects via the induction of signal transduction pathway involving JAK 1 & 2 and STAT1. The binding of IFNγ to its surface receptor activates the receptor-associated tyrosine kinases, JAK1 and JAK2. JAKs tyrosine phosphorylate and activate the latent cytosolic STAT1, which then dimerizes, translocates to the nucleus [108] where it binds to IFNγ-response elements [109, 110] IFNγ decreased DRA promoter activity in Caco2 cells via a JAK/STAT1 pathway [100]. Progressive deletions from the 5′-flanking region of DRA promoter showed that the IFNγ-responsive region was located between −1183 to −790 bp region [100]. Sequence analysis of this region identified one potential GAS cis element flanking the region of −933 to −925 bp. GAS element is a 8- to 10-bp inverted repeat DNA element with a consensus sequence of $5'\text{-TT(N}_{4-6})\text{ AA-3}'$[109]. Additionally, EMSA showed increased protein binding to the oligonucleotide spanning the potential GAS element (−933/−925 bp) in response to IFNγ, which was blocked by STAT1 antibody, suggesting that the identified protein is STAT1 [100]. Also, mutational studies and *in vivo* ChIP assays showed that GAS element is required for the inhibitory effects of IFNγ on DRA promoteractivity. These findings provided evidence for the role of STAT1 in the inhibition of DRA promoter activity by IFNγ [100] (Figure 4). Increased expression and activation of STAT1 has been observed in the colonic mucosa of patients with UC and to a lesser degree in CD. [111]. Infact, the anti-inflammatory effects of glucocorticoids have been suggested to occur via inhibition of STAT1 in IBD patients [111]. Therefore, the development of novel therapeutic modalities against STAT1 may be of importance in the treatment of colonic inflammation where DRA expression and function are downregulated.

In contrast to the role of STAT1 in IFNγ-mediated effects on DRA promoter activity, PAT1 promoter activity was also decreased by IFNγ albeit via distinct cis element/transcription factor [101]. Progressive deletions from the 5′-flanking region of PAT1 promoter showed the presence of potential IFNγ response element (s) region between −414 to −214 bp [101]. Sequence analysis of the IFNγ responsive region identified one potential ISRE (Interferon Stimulated Response Element) binding site flanking the region of −318 to −300 bp. The ISREs, usually defined by pallindromic TTTC sequences separated by two or three nucleotides are located in the promoter region of many IFNγ inducible genes [110]. EMSA also showed increased protein binding to the oligonucleotide spanning the potential ISRE site (−318/−300 bp) in the presence of IFNγ. Additionally, supershift assays with antibody

against IRF-1 transcription factor showed that the DNA-protein binding was blocked indicating that IRF-1 binds to the ISRE site [101]. This was further confirmed by ChIP assays showing that the association of IRF-1 with endogenous PAT1 promoter (containing the ISRE site) was significantly enhanced by IFNγ. Mutations in the potential ISRE cis element completely attenuated the inhibitory effects of IFNγ on promoter activity further suggesting the involvement of IRF-1 in the regulation of PAT1 promoter activity by IFNγ [101] (Figure 4). IRF-1 expression is increased in the lamina propria of patients with active Crohn's disease [112]. Since, CD commonly affects the ileum, it is plausible to speculate that IRF-1 plays a key role in the inflammation of ileum. Immunosuppressive treatment (azathioprine or 6-mercaptopurine for 24h) of patients with CD has been shown to reduce IRF-1 [112]. Although, the role of PAT1 in intestinal inflammation has not been established, targeting of IRF-1 as a therapeutic alternative may be important in the treatment of ileal inflammation where PAT1 expression and function (Cl^- absorption) are inhibited.

CONCLUSIONS

The advent of cloning and the use of transgenic mouse models have significantly enhanced our understanding of the molecular mechanisms underlying NaCl absorption and its regulation in health and disease. It is now accepted that NHE2, NHE3 and DRA, PAT1 are the predominant luminal membrane Na^+ and Cl^- absorbing proteins involved in vectorial NaCl absorption in the adult mammalian intestine. Recent studies have also provided strong evidence that in addition to short-term regulation of these transporters, long-term regulation has strong implications in tissue, region and differentiation specific expression of these genes under physiological conditions. Studies from disease models and patients have further shown that repression of NHE3 and DRA expression are associated with infectious diarrhea or inflammatory bowel diseases. Until recently, detailed mechanistic understanding of the long-term regulation of these transporters was very limited. However, with cloning of the promoters of these transporters, new data on their transcriptional regulation has now started to emerge. These data have identified and characterized the core promoter, the predicted TF binding sites and the TFs involved in basal transcription and regulation of these genes by various physiological and pathophysiological agents. These studies demonstrate that basal transcriptional regulation of both the human NHE2 and NHE3 involves transcription factors Sp1 and Sp3. Although, AP2 was also found to interact with NHE3 promoter, the functional significance of this binding remains to be investigated. The studies further demonstrated that diverse effector molecules e.g. butyrate, phobol esters, IFNγ, TNFα and serotonin converge at a site containing the overlapping Egr-1/Sp1 motif in NHE3 promoter to trigger distinct outcomes. However, the detailed molecular mechanisms as to how these agents exert distinct effects on NHE3 promoter remains to be investigated. In contrast to NHE3, NHE2 repression by inflammatory cytokines was found to involve activation of the NFκB pathway. It is, however, not clear whether NFκB represses NHE2 promoter activity via direct binding or through a putative downstream transcription factor. With respect to transcriptional regulation of DRA and PAT1, the available data is even more sketchy. This has been mainly due to identification of their role in intestinal chloride absorption only very recently and the cloning of their promoters was accomplished only during the last 3 years. It is now known that HNF4 is involved in basal transcription of DRA, however, its stimulation by butyrate involved GATA and YY1 factors. However, the exact GATA isoform involved in the regulation of DRA promoter and the interplay between GATA and YY1 needs more extensive investigation. The basal transcriptional regulation of PAT1 has not been characterized, however, sequence analysis of the promoter region predicted that HNF1α, YY1 and Sp1 might play important roles. Studies have further shown that the cytokine IFNγ down-regulated the expression of both the DRA and PAT1 genes, albeit, via distinct mechanisms. While the activation of STAT1 via JAK-STAT pathway and its direct binding to GAS element mediated the effect of IFNγ on DRA promoter, the activation of IRF-1 by

STAT1 and subsequent binding of IRF-1 to ISRE was involved in the suppression of PAT1 promoter by IFNγ. Repression of both PAT1 or DRA and NHE3 promoter activities in response to IFNγ may lead to decreased NaCl absorption in the ileum and colon. The overall decrease in NaCl absorption may play a crucial role in the pathophysiology of diarrhea associated with IBD. Taken, together, these data indicate that down-regulation of NHE2, 3 and DRA and PAT1 expression and the decrease in the promoter activities in response to inflammatory mediators may contribute to pathophysiology of diarrhea associated with inflammatory disorders of the gut. On the other hand, mechanisms involved in the induction of gene expression of NHE3 or DRA by agents e.g. butyrate, soluble factors secreted by probiotics or glucocorticoids need to be investigated in detail as these could be exploited to improve the treatment modalities of the inflammatory bowel diseases. Furthermore, a detailed characterization of the newly identified NHE8 may also add more to our understanding of NaCl absorption and its regulation in health and disease as well as in developing and adult intestine. Finally, the role of epigenetic mechanisms in regulating the expression of intestinal Na^+ and Cl^- transporters is not fully understood and warrants future investigations.

Acknowledgments

ABBREVIATIONS USED

5-HT	5-hydroxytryptamine
AP	activator protein
C2BBE1	subclone of Caco2 cells
Caco2	human colon adenocarcinoma
Cdx2	caudal-related homeobox 2
CLD	Congenital Chloride Diarrhea
CREB	cAMP response element binding
DRA	Down Regulated in Adenoma
EGF	epidermal growth factor
Egr-1	early growth response protein 1
ERK1/2	extracellular signal regulated kinase 1/2
GAS	γ-activated site
GRE	glucocorticoid response element
h	human
HEK293	human embryonic kidney
HepG2	human hepatocellular liver carcinoma
HNF	hepatocyte nuclear factor
IBD	Inflammatory Bowel Diseases
IBS	Irritable Bowel Syndrome

IEC	intestinal epithelial cell
IFNγ	interferon γ
IL1β	interleukin 1β
IRF-1	interferon regulatory factor 1
ISRE	interferon stimulated response element
JAK	janus kinase
LLC-PCK1	pig kidney epithelial
LS174T	human colon cancer
Luc	luciferase
MAPK	mitogen-activated protein kinase
MAZ	myc-associated zinc finger
MyoD	myogenic differentiation 1
Mzf1	myeloid zinc finger 1
NCM460	normal human colon mucosal epithelial
NFB	nuclear factor kappa B
NHE	sodium hydrogen exchanger
NIH3T3	mouse embryonic fibroblast
Oct-1	octameric transcription factor 1
OK	opossum kidney
PAT1	Putative Anion Transporter 1
PKA	protein kinase A
PKC	protein kinase C
PMA	phorbol 12 myristate 13-acetate
PRE	PMA response element
PS120	Chinese hamster lung fibroblast
QT-6	avian (quail origin-6)
r	rat
SL2	schneider cell line 2
SLC	solute carrier
Sp	specificity protein
STAT	signal transducer and activator transcription factor
T3R	thyroid hormone receptor
TF	transcription factor
TIS	transcription initiation site
TNFα	tumor necrosis factorα
USF	upstream stimulatory factor

VDR	vitamin D3 receptor
YY1	Ying Yang 1
ZBP-89	zinc-binding protein-89

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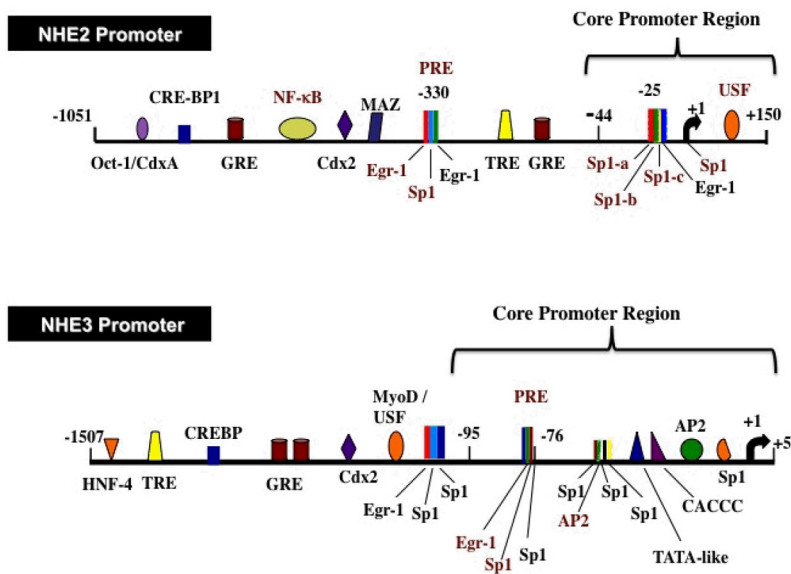
Figure 1. Schematics of the human NHE2 and NHE3 promoters
Predicted protein binding sites are indicated. Experimentally verified functional protein binding sites are shown in red text.
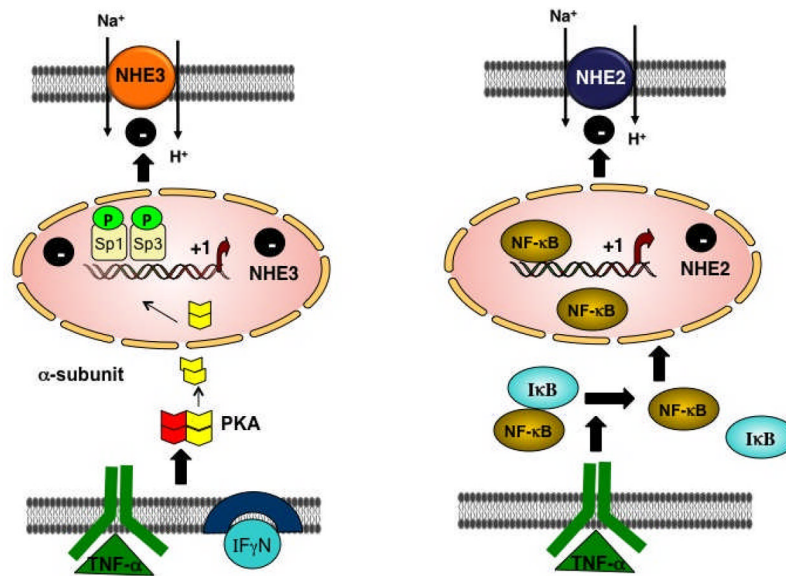
Figure 2. Potential mechanisms regulating the human NHE2 and NHE3 expression by pro-inflammatory cytokines in C2BBE1 cells: NHE2

TNFα-mediated degradation of IκB-αin the cytoplasm and subsequent activation and translocation of NFκB to the nucleus results in its interaction with the NHE2 promoter. This leads to repressed NHE2 transcription and reduced levels of NHE2 mRNA, protein and function. **NHE3.** A combination of IFNγ/TNFαrepresses the NHE3 gene expression by PKA activation and translocation of its catalytic subunit to the nucleus where it phosphorylates the Sp1/Sp3. As a result, binding of Sp1/Sp3 to the promoter region is decreased leading to reduced NHE3 transcription, mRNA and protein expression and its function.
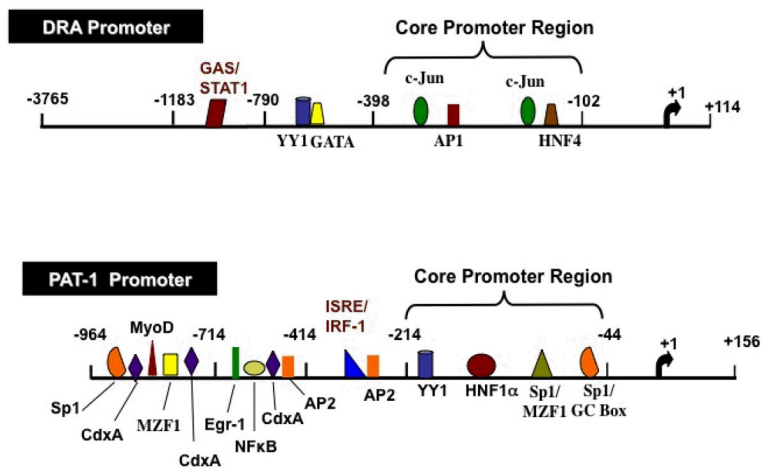
Figure 3. Schematics of the human DRA and PAT1 promoters
The transcription initiation site is marked as +1. Predicted transcription factor binding sites are indicated. Transcription factors shown in red text indicate IFNγ response elements and in blue text indicate butyrate response elements.
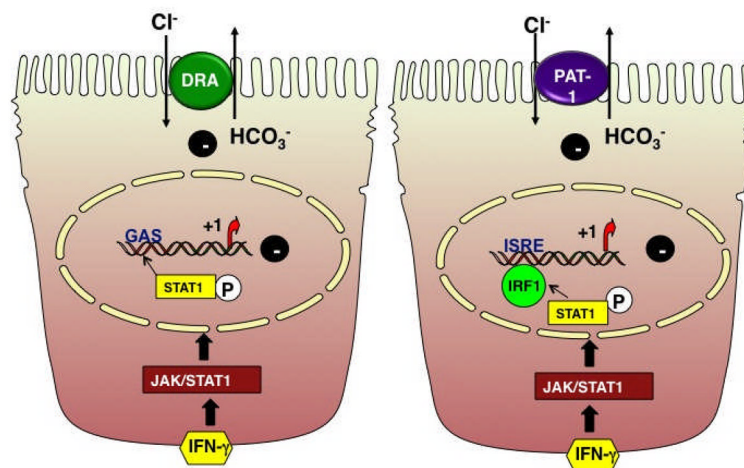
Figure 4. Proposed model for inhibition of DRA and PAT-1 promoter activity by IFNγ
IFNγ binds to the IFNγ-receptor to activate JAK 1 & 2. Activated JAK 1 & 2 phosphorylate the latent cytoplasmic STAT1, which gets activated, dimerizes and is translocated to the nucleus. In the nucleus, phospho STAT1 binds to the GAS element of DRA promoter or activates IRF-1 that binds to ISRE site of PAT-1 promoter, which in turn leads to a decrease in DRA or PAT-1 mRNA, protein expression and function.

Table 1

Modulation of NHE2, 3, 8, DRA and PAT-1 Expression by Various Stimuli.

Stimuli	NHE2	NHE3	NHE8	DRA	PAT-1
Butyrate	NE (67)	↑ (55,67,68,69)	ND	↑ (103)	ND
EGF	↑ (33,75)	NE (75)	↓ (76)	ND	ND
Glucocorticoids	NE (70)	↑ (51,52,70)	↓ (74)	ND	ND
PMA	↑ (37)	↑ (53)	ND	ND	ND
TNF	↓ (33)	↓ (54)	↓ (62)	ND	ND
IFN-γ	↓ (81)	↓ (54,81)	ND	↓ (100)	↓ (101)
IL1-β	ND	ND	ND	↓ (96)	ND
Serotonin	ND	↓ (56)	ND	ND	ND
L. acidophilus	ND	ND	ND	↑ (102)	NE

ND: Not determined, NE: No effect. Arrows indicate the direction of change. Numbers in parentheses indicate the reference numbers.